

THE TRANSMISSION OF *T. CONGOLENSE* BY *GLOSSINA* *PALPALIS*

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I. INTRODUCTION

Representatives of the group of trypanosomes to which *T. congolense* and *T. nanum* belong are widespread throughout Africa. They are common alike in game and in stock. To the game they are, apparently, harmless, but as parasites of domestic animals they constitute a factor of considerable economic importance.

The trypanosomes of this group are well adapted to rapid propagation in nature. For indirect or cyclical transmission they depend, so far as is known, entirely on the *Glossinae*; and, though less common than *T. vivax* and *T. uniforme* in wild game-tsetse, they are considerably more prevalent in the fly than the polymorphic trypanosomes of the *T. brucei* group. In all species of *Glossinae* in which cyclical development of the *T. congolense* group takes place, the flagellates multiply first in the gut of the fly, and later on take up their 'anterior station' in the labrum and hypopharynx of the proboscis.

In addition to cyclical transmission, they are readily conveyed

from mammal to mammal by the direct method—that is, without any alternation of generations. It is probable that most, if not all, the mammalian trypanosomes can, on occasion, avail themselves of direct transmission, but it is doubtful whether any other group of the *Glossina*-carried organisms relies to the same extent on this method in nature.

The so-called species contained within this group have been arbitrarily separated on account of differences in their behaviour in certain mammals. The *nanum*-form cannot infect dogs and monkeys and the small laboratory animals, while the *congolense*-form can; otherwise they are indistinguishable. Both these two forms of the trypanosome have been recovered from game and from wild fly, so that this idiosyncrasy may, to a slight extent, influence the distribution of the parasites in certain regions. But to allow to physiological variations of this kind the importance attaching to a specific character, is, as Yorke and Blacklock (1914) have pointed out, unjustifiable; and, as a matter of fact, strains whose virulence is intermediate between these two extremes do occur in nature. The group should, therefore, be regarded as a collection of strains whose morphology and behaviour in the insect intermediary are uniform and constant, but which vary in their relation to certain mammalian hosts. It has been shown elsewhere by laboratory experiments, that the effect of long-continued direct transmission on a strain of *T. brucei* may be to enhance its virulence in mammals, and, at the same time, to eliminate the power of developing cyclically in *Glossinae*. The same principles probably apply to the group we are considering. There is, indeed, field-evidence to show that some of the more virulent strains of *T. congolense* rely largely, if not solely, on direct transmission, for their passage from mammal to mammal. Whether such strains retain the power of cyclical development in *Glossinae* has, however, not yet been determined.

The gregarious habits of domestic stock afford excellent opportunities for the spread of their trypanosomes by direct transmission, by *Stomoxys* and the other species of biting flies that, in Central Africa, swarm around the unfortunate animals. Among the game this method functions probably much less commonly, although with certain species such as elephant, buffalo and eland the requisite conditions will doubtless often occur.

However this may be, there is an essential difference between game and stock in their relation to these parasites. For while the game is not, so far as is known, in any way inconvenienced even by double infections of the trypanosomes indigenous to its habitat—whether directly or cyclically transmitted—cattle, as a rule, cannot survive even brief contact with the game-tsetse, and are particularly sensitive to strains of *T. congolense* in non-tsetse areas. In localities where game-tsetse are present and game plentiful, a certain percentage of the wild fly will always be found to carry the developmental forms of the proboscis-and-gut group of trypanosomes. In *G. palpalis* areas, however, matters are different. The commonest flagellate found in this fly in nature is probably *T. grayi*. Of the mammalian trypanosomes, the proboscis-only group is most commonly found, represented by *T. vivax* and *T. uniforme*; the gut-and-gland group, usually in the form of *T. gambiense*, is also of common occurrence; but the proboscis-and-gut group is distinguished by the comparative rarity with which it develops in wild *G. palpalis*.

So far as I can determine, three instances are recorded of the occurrence of developmental stages of the proboscis-and-gut group in wild *G. palpalis*. These are as follows:—Warrington Yorke and Blacklock (1915), investigating an epidemic of cattle trypanosomiasis in Sierra Leone, found nineteen out of one hundred and forty-seven cattle infected with trypanosomes; *T. congolense* was found in sixteen, *T. vivax* in seven, and *T. gambiense* in one. In the course of the dissection of four hundred wild *G. palpalis* caught in the same neighbourhood, proboscis-only infections were found in fifteen flies, gut-only in two, and gut-and-proboscis in four.

Macfie (1915) dissected seventy-five wild *G. palpalis* from near Accra, and found eleven infected with flagellates—three gut-and-gland, three proboscis-only, and one proboscis-and-gut. In the review of these experiments, there follows the seemingly contradictory comment that 'none of these infections resembled stages in the development of *T. congolense*.' Possibly the flagellates in the proboscis of the single gut-and-proboscis fly were not fixed, and, in consequence, were not regarded as true proboscis-forms.

The third instance occurred in my own work (1916) in the Northern Province of Uganda, where, in two areas, proboscis-

and-gut infections of wild *G. palpalis* were found, amounting in one case to 1.4 per cent. and in the other to 5.2 per cent. of the flies dissected. Allowing for the possible occurrence in some of these flies of double infections, the last figure is sufficiently high to prove conclusively that, in this area, the *congolense-nanum* type of trypanosome is carried cyclically by *G. palpalis*.

In the face of these examples it is the more remarkable that no instance has hitherto been recorded of the occurrence of this group of trypanosomes in the wild *G. palpalis* of the Victoria Nyanza. At many points along the shores of this great lake, cattle came in contact with this tsetse; and there are long stretches of shore in Busoga where game and *G. pallidipes* occur immediately behind the *G. palpalis* shelter, and where the latter fly has plenty of opportunity for picking up parasites of the *T. congolense* group. *T. vivax*, *T. uniforme*, and a member of the gut-and-gland group are all found in these lakeshore *G. palpalis*. During the fourteen odd years that have elapsed since the depopulation of the Sesse Islands, the Situtunga have multiplied enormously, and the tsetses on these Islands have come to rely on the antelope to a great extent for food. The three trypanosome species just mentioned, are common in these Situtunga, but on no occasion has the *T. congolense* type been found in their blood. Apparently *G. palpalis*, in its natural environment, is not, as a rule, suited to the cyclical transmission of these trypanosomes.

A possible explanation of the absence of the proboscis-and-gut group from the fly on the Victoria Nyanza is, that the strains with which the fly come in contact are directly transmitted strains, which have lost the power of cyclical development in *Glossinae*. But this explanation alone is inadequate to account for the absence of these parasites from this huge fly area.

II. PREVIOUS WORK ON THE TRANSMISSION OF THE PROBOSCIS-AND-GUT GROUP OF TRYPANOSOMES BY *G. PALPALIS*

The Royal Society's Commission in Uganda (1910) experimented with wild and with laboratory-bred flies. Two successful transmissions were reported. In one experiment, with wild *G. palpalis*, the flies became infective to a clean animal twenty-one

days after the first infecting feed; in the other, in which laboratory-bred flies were employed, the flies were infective on the fourteenth day of the experiment. The authors remark that these last experiments were open to fallacy, as an epidemic of trypanosomiasis, due to a member of the 'proboscis-and-gut' group, was occurring at the time in the neighbourhood of the laboratory. I think it probable that the same explanation applies to the wild fly transmission also. No positive flies were found on dissection of the flies in these experiments, and at the time, it was not known that the developmental cycle of *T. congolense* included an invasion of the proboscis of the fly. Subsequent experience has shown it to be improbable that the trypanosomes of this group can complete their cycle in *G. palpalis* in such a short time as twenty-one days.

Of the attempts by the same Commission to transmit *T. nanum* by *G. palpalis*, we read that the only experiment attempted was 'unsatisfactory, as trypanosomes appeared in the first healthy goat a few days after the fly had fed on infected animal,' *i.e.*, it was a natural infection due to some agency outside the experiment.

In view of the ease with which these 'proboscis-and-gut' trypanosomes are propagated in nature by agents other than tsetse-flies, it is, in my opinion, unsafe to carry out experiments at a spot where the disease is already existent, especially if ruminants are employed to demonstrate the transmission.

In 1911, Fraser and Duke carried out seven transmission experiments with laboratory bred flies. One experiment was successful, trypanosomes appearing in the blood of the clean monkey on the eighty-fifth day after the first infecting feed of the flies. On the ninety-sixth day of the experiment a fly died which showed a heavy proboscis-infection. This fly, after infecting the clean monkey, had had access to two other clean monkeys, upon one of which it certainly fed several times. Neither of these last two animals became infected. At the time, it was supposed that the insect must have lost its infectivity, but I now believe that the explanation lay in the different resistance of the host-animals.

In the dissections of these experiments, twenty positive flies were found in a total of four hundred and twenty-seven dissected. Among these were five flies with flagellates established in the proboscis. One of these infected the monkey in the positive

experiment. Two occurred in another experiment, and fed repeatedly on a clean monkey without causing infection; they never had access to a ruminant animal. The remaining two flies with proboscis-infections occurred in a third experiment, and died on the fifty-ninth and seventy-fourth days after the original infecting feed; they both fed repeatedly on a clean monkey without ever infecting it.

The contents of the proboscis of all these five positive flies were injected into rats, without infection resulting. In these experiments no infection of the proboscis was met with in flies dissected before the fiftieth day after the first infecting feed. The salivary glands of these positive flies were all negative to flagellates. In all these experiments the clean animal employed was a monkey. No trypanosome disease existed in the vicinity of the laboratory at the time.

In 1911-12 experiments were carried out on the transmission of *T. nanum* by laboratory-bred *G. palpalis* in Uganda. In the first set of experiments a goat was used for the infecting feeds; one hundred and seventy-three flies were dissected during these experiments; no infected flies were obtained and no transmission occurred.

In the next series of experiments an infected sheep was employed, on which the flies fed much more readily, and the parasite was transmitted to a clean calf. Three hundred and twenty-two flies were dissected, of which twelve were infected, five showing flagellates established in the proboscis. In only one of the proboscis-infections were flagellates seen in the hypopharynx; the labrum of this fly contained great clusters, while a few individuals were present in the hypopharynx. This fly died on the twenty-fifth day after the first infecting feed—the earliest recorded date for the infection of the proboscis of *G. palpalis* by a member of this group of trypanosomes.

In 1911-12 another series of transmission experiments were carried out with *T. congolense* and laboratory-bred *G. palpalis*. The animal used for infecting the flies was a young bushbuck which had been born at the laboratory and, when a few months old, had been infected by syringe inoculation with the Mpumu Laboratory strain. In the course of these experiments, seven hundred and forty-six flies were dissected, of which six hundred and thirteen lived

until the thirtieth day after their first feed on infected blood. Fifteen flies were found to contain flagellates; four had proboscis-infections; and one a heavy infection of the sucking-stomach, with no flagellates in the proboscis. The box which was apparently responsible for the successful transmission, contained this fly with the infected sucking-stomach and none with infected proboscis. The four flies with infected proboscides died respectively on the seventy-sixth, one hundred and fourth, and one hundred and forty-first days after the first infecting feed. A ninetieth day fly showed flagellates in the hind-gut only.

It must be noted that the strain of *T. congolense* kept at the Mpumu Laboratory was derived from cattle, from localities where tsetse-flies are absent or scarce. It is, therefore, probable that it was a directly transmitted strain, before it commenced its career at the laboratory. Too much stress, therefore, cannot be laid upon its behaviour when exposed to tsetses.

The strain of *T. nanum*, on the other hand, came from cattle in the *G. pallidipes* country in Toro Kingdom, and Sheep Experiment 59, by which the flies were infected, was the second passage animal from the original ox.

As already stated, the strain of *T. congolense* used in the experiments now to be set forth is known to be a cyclically carried wild fly strain.

III. HISTORY OF THE TRYPANOSOME STRAIN EMPLOYED IN THE TRANSMISSION EXPERIMENTS PERFORMED AT ENTEBBE DURING 1922 and 1923

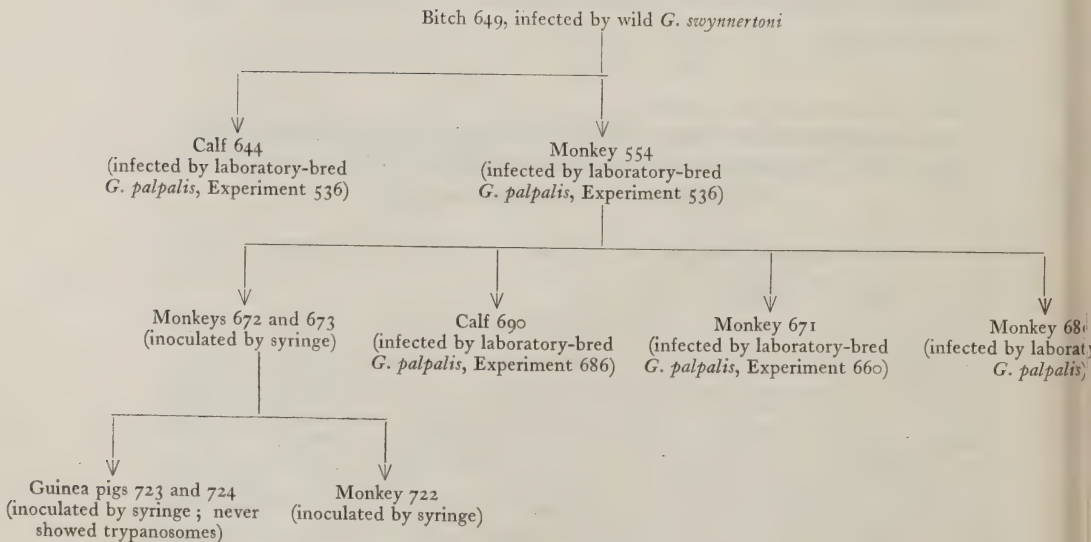
This strain was brought back from Mwanza, in August, 1922, in Bitch Experiment 649.

On August 1st, 1922, Experiment 649 was fed upon by a batch of wild *G. swynnertoni*, of which two, on subsequent dissection, were found to have proboscis-and-gut infections, the salivary glands being negative. On August 8th, 1922, a single trypanosome of the *T. congolense* type was seen in the animal's blood. On August 9th, 10th and 11th, the blood was negative to fresh film examination; on the 12th a few trypanosomes were seen, and thereafter the animal was negative to daily examination of stained thick films

until September 13th. From this date trypanosomes were found at frequent intervals in the course of the daily routine examinations, until February 2nd, 1923. Then followed a series of negative examinations until February 28th, when a few parasites were seen in a stained thick film. Trypanosomes were next seen, on a single occasion only, on August 20th, 1923. For a month or so after arriving at the Entebbe Laboratory, the animal was languid and seemed to be losing condition. In October she showed signs of being pregnant; the mammae enlarged and the animal became fatter, but in a week or two these symptoms disappeared. The general condition was now excellent. She became pregnant about the middle of June, and on August 24th, 1923, at 12 o'clock, two healthy puppies were born, followed, an hour and a half later, by a third which died an hour after birth.

The subsequent upkeep of the strain from this bitch is shown in Table I.

TABLE I.



IV. ANIMAL REACTIONS OF THE STRAIN OF *T. CONGOLENSIS* REFERRED TO IN THIS PAPER

The *T. congolense* isolated by Bruce's Commission and employed in their original transmission experiments at Mpumu, lost some of its virulence during two years of maintenance at that laboratory. The average duration of the disease in ten completed monkey experiments carried out by Bruce was sixty-three days; one animal died after one hundred and eighty-one days, the average for the other nine being forty-eight days. Another monkey was alive after two hundred and sixteen days. In eight completed dog experiments the average duration was forty-three days. Of three monkeys inoculated with the same strain eighteen months later, one died after thirty-five days, one after one hundred and fifty days, and the other was still alive after two hundred and three days.

The animal reactions of the strain from the Mwanza Fly Belt are as follows:—

TABLE II.

	Incubation period, in days					Duration of disease, in days
Monkeys—						
554	78 days or more	127 at least.	
671	47 days or more	Still alive after 145 days.	
689	?			47th day after first appearance of trypanosomes of Fly Experiment 686.	
672	16	Still alive after 188 days.	
673	16	64 (trypanosomes swarming in blood).	
722	38	43 (trypanosomes swarming in blood).	
Calves—						
640	10	Alive and apparently well after 7 months.	
644	8	Alive and apparently well after 9 months.	
690	23-25	Alive and apparently well after 107 days.	

V. TECHNIQUE OBSERVED IN THE CONDUCT OF THESE TRANSMISSION EXPERIMENTS

The transmission experiments were carried out according to the methods pursued for many years in the Uganda Laboratory. The newly hatched flies are placed in wire-sided boxes and fed and starved on alternate days, dead flies being removed for dissection each morning. During the act of feeding, a wet rag covers the top side of the box, while the other side is closely applied to the animal's skin. Feeding continues until the flies have had all the blood they want. At the termination of the experiment the survivors are killed with chloroform vapour and dissected. Experience has proved that the rough and ready method of holding the box in the smoke of a fire, though effective in killing the flies, also kills the flagellates they contain, and makes the identification of light infections more difficult. Throughout the experiments the boxes are kept on stones resting in dishes of clean water.

Whenever a fly with a proboscis-infection was found, the animals upon which the insect had fed were examined daily by means of a stained thick blood-film. Otherwise, all experimental animals were examined daily by careful inspection of fresh unstained blood-films.

VI. THE TRANSMISSION EXPERIMENTS

The actual experiments will now be set forth. In the 'positive-flies' column the contents of the brackets refer to the number of flies with infected proboscides. In the 'remarks' column will be found the result of the experiment, positive or negative, according as the clean animals develop trypanosomes or not.

(A) *Experiments in which some of the flies developed proboscis-infections.*

(1) Experiments in which cyclical transmission of *T. congolense* from sick to healthy animal occurred.

TABLE III.
EXPERIMENT 536.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1922 Sept. 25-27	1-3	Flies fed on bitch 649	<i>T. congolense</i> not seen in stained thick films of bitch's blood.
" 28	4	Starved	
" 29-Nov. 21	5-58	Fed on clean monkey 554	2 (1 proboscis +)	<i>T. congolense</i> first seen in 554's blood on 13 Feb., 1923.
Nov. 22	59	Starved	
" 23-Dec. 1	60-68	Fed on clean calf 644 and on 554 on alternate days	...	<i>T. congolense</i> first seen in calf's blood on Dec. 1.
Dec. 2	69	Starved	
" 3-4	70-71	Fed on clean monkey 611	...	Monkey 611 never showed trypanosomes.
" 5	72	Remaining 28 flies dissected	3 (1 proboscis +)	

Flies dying before 16th day were ignored.

Total number of flies dissected during the experiment = 68.

Number alive on 25th day of the experiment = 64.

Remarks. November 27th was the last date on which the flies had access to 554, the incubation period in the monkey was thus very long, amounting to at least seventy-eight days. In contrast to this the incubation period in the calf was only nine days.

TABLE IV.
EXPERIMENT 660.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1923 Feb. 16	1	Flies fed on monkey 554	...	<i>T. congolense</i> present in 554's blood.
" 17	2	Starved	
" 18-19	3-4	Fed on monkey 554	<i>T. congolense</i> present in 554's blood.
" 20	5	Starved	
" 21-23	6-8	Fed on monkey 554	<i>T. congolense</i> present in 554's blood.
" 24	9	Starved	
" 25-April 8	10-52	Fed on clean monkey 671 on alternate days	1 (proboscis +++) 51st day	Monkey 671 first showed <i>T. congolense</i> on 24 May, 1923
April 9-10	53-54	Starved	
" 11	55	Remaining 19 flies dissected	2	

Flies dying before the 22nd day of the experiment were ignored.
Total number of flies dissected = 58.
Number alive on 25th day of experiment = 55.

Remarks. Note the long incubation period of *T. congolense* in monkey 671, *i.e.*, from, say, the first week in April until 24th of May.

TABLE V.
EXPERIMENT 686.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1923 Mar. 11-13	1-3	Flies fed on monkey 554	...	<i>T. congolense</i> present in 554's blood.
" 14-15	4-5	Starved	
" 16-May 13	6-64	Fed on clean monkey ...	1 (proboscis +) 54th day	Monkey 689 first showed <i>T. congolense</i> in blood on 23 May, 1923.
May 14	65	Starved	
" 15-17	66-68	Fed on clean calf 690	Calf 690 first showed <i>T. congolense</i> in blood on 7 June, 1923.
" 18-19	69-70	Starved	1 (proboscis +) 69th day	
" 20	71	Fed on 689	
" 21	72	Starved	
" 22	73	Fed on 689	
" 23-24	74-75	Starved	
" 25	76	Remaining 14 flies dissected	...	

Flies dying before 12th day were ignored.
Total number of flies dissected = 49.
Number alive at 25th day = 46.

Remarks. It is impossible to estimate the incubation period in monkey 689. In the calf, trypanosomes appeared twenty-three to twenty-five days after the infecting feed.

(2) Experiments in which no transmission occurred.

TABLE VI.
EXPERIMENTS 597-8.

Date	Day of Experiment	Procedure	Positive Flies found	Remarks
1922 Nov. 18	1	Flies fed on dog 649	<i>T. congolense</i> not seen in dog's blood.
" 19	2	Starved	
" 20-21	3-4	Fed on dog	<i>T. congolense</i> not seen in dog's blood.
" 22	5	Starved	
" 23-Dec. 25	6-38	Fed alternate days on clean monkey 602	...	Monkey 602 never showed trypanosomes: examined by thick stained films till 15 June, 1923.
Dec. 26	39	Starved	
" 27	40	Fed on clean dog X	Dog X never showed trypanosomes.
" 28	41	Starved	
" 29	42	Fed on dog X	
" 30	43	Starved	2 (1 proboscis +)	This fly had fed on dog X.
" 31-Jan. 16	44-60	Fed on alternate days on monkey 602 and dog X	...	
Jan. 17	61	Remaining 32 flies dissected	...	

Flies dying before the 11th day of the experiment were ignored.
 Total number of flies dissected = 113.
 Number alive at 25th day of experiment = 105.

Remarks. Neither of the clean animals of this experiment became infected. The infection of the proboscis in the forty-three day fly was not heavy, and may have been established after the fly had been removed from contact with monkey 602.

Possibly, in the earlier stages of the invasion of the proboscis, the fly is only capable of infecting very susceptible mammals, on account of the small number of trypanosomes which it inoculates.

TABLE VII.
EXPERIMENT 658.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1923 Feb. 15-16	1-2	Fed on monkey 554	<i>T. congolense</i> present in 554's blood.
" 17	3	Starved	
" 18	4	Fed on monkey 554	<i>T. congolense</i> present in 554's blood.
" 19	5	Starved	
" 20	6	Fed on monkey 554	<i>T. congolense</i> present in 554's blood.
" 21	7	Starved	
" 22	8	Fed on monkey 554	<i>T. congolense</i> present in 554's blood.
" 23	9	Starved	
" 24-April 21	10-66	Fed on clean monkey 668	1 (proboscis +) 66th day	Monkey never showed trypanosomes: examined daily, stained thick films till 23 July, 1923.
April 22	67	Starved	
" 23-25	68-70	Dog T	Dog never showed trypanosomes.
" 26	71	Starved	
" 27-May 1	72-76	Fed on monkey 668 and starved alternate days	...	
May 2-3	77-78	Fed on clean calf	Calf never showed trypanosomes.
" 4-6	79-81	Starved	
" 7	82	Remaining 12 flies dissected	...	

Flies dying before the 17th day were ignored.
Total number of flies dissected = 41.
Number alive on 25th day = 47.

Remarks. The only positive fly of this experiment fed repeatedly on monkey 668 without causing infection. Unfortunately this fly died before feeding on the dog or the calf.

(B) *Experiments in which no flies with infected proboscides were found.*

(In none of these experiments did transmission occur.)

Table VIII gives a summary of these experiments.

TABLE VIII.

Experiment	Date of infecting feeds	Infecting animals	NUMBER OF FLIES			Duration of Experiment in days	Day of Experiment on which dissection began
			Alive 25th day	Dissected during Experiment	Containing flagellates in gut		
520	19-21.9.22 ...	Bitch 649 ...	59	63	0	77	18th
600-1	22-24.11.22 ...	Bitch 649 ...	85	123	0	57	13th
643	9, 10 and 12.2.23	Calf 644 ...	58	66	0	44	16th
662	18-21.2.23 ...	Calf 644 ...	40	54	2	68	13th
665	21-23.2.23 ...	Calf 644 ...	37	37	0	65	24th
664	19-21 and 23.2.23	Monkey 544 ...	46	50	0	77	18th
667	23-25.2.23 ...	Calf 644 ...	53	63	0	61	11th
674	1-3.3.23 ...	Calf 644 ...	39	46	0	74	15th
678	3-6.3.23 ...	Calf 644 ...	46	47	0	72	19th
684	8-10.3.23 ...	Monkey 554 ...	29	31	0	78	16th
693	15-17.3.23 ...	Monkey 673 ...	50	53	1	83	19th
700	19-22.3.23 ...	Monkey 554 ...	44	53	0	82	14th
			586	686	3		

The results of dissection of the positive flies of all the above experiments are shown in Table IX.

TABLE IX.

Experiment	Number of fly	Day of dissection reckoned from first infection feed	Distribution of flagellates		Remarks
			Gut	Proboscis	
693	1	20th day	+++	o	
662	2	21st day	+++	o	
536	3	26th day	+++	o	
543	4	34th day	+++	o	
579-8	5	43rd day	+++	o	
...	6	43rd day	+++	+	
660	7	51st day	+++	+++	
543	8	52nd day	+++	+++	
662	9	53rd day	+++	o	
686	10	54th day	+++	++	
660	11	55th day	+++	o	
...	12	55th day	+++	o	
536	13	57th day	+++	+++	Sucking stomach +++
642	14	58th day	+++	o	
536	15	62nd day	+++	o	
658	16	66th day	+++	+++	
686	17	69th day	+++	++	
536	18	72nd day	+++	o	
...	19	72nd day	+++	+++	
...	20	72nd day	+++	+++	

VII. DISCUSSION OF THE EXPERIMENTS

It will be seen from Table IX that the earliest date at which flagellates were found in the proboscis was in a forty-three day fly. It is, however, possible that the invasion of the proboscis in some of the older flies took place earlier than this. On the other hand,

several infected flies lived considerably longer than forty-three days without the flagellates reaching the 'anterior station.'

The experiments of Group A show that flies with heavily infected proboscides may feed upon a clean animal without causing infection. It is interesting to note that, when this happened, in each case the clean animal was either a monkey or a dog: whenever a calf was bitten by a fly with an infected proboscis, the animal developed trypanosomes.

It was hoped, by exposing, alternately, different species of clean animals to the infected flies of these experiments, to throw light upon the biological significance of the differences in virulence and of host proclivities shown by the trypanosomes of this group. But, unfortunately, on several occasions the infected fly died before the box was transferred to a second, or a third clean animal. It was thus not possible to ascertain whether a fly may be infective to a ruminant and yet be unable cyclically to infect a monkey or a dog.

The strain of *T. congolense* used in these experiments was of comparatively low virulence. On several occasions the incubation period in monkeys, before trypanosomes appeared in the peripheral blood, was very long. In the case of monkeys Nos. 554 and 671, both of which were infected by flies, the incubation periods were seventy-eight and forty-seven days, respectively; with monkey Experiment 722, infected by the syringe, thirty-seven days elapsed before trypanosomes were detected. The incubation periods in the calves infected by flies were nine and twenty-three to twenty-five days. Similarly, in my experiments at Mpumu, the incubation period in the only monkey infected by cyclical transmission was thirty-three days, while in another monkey, infected by means of the syringe, the incubation period was forty-seven days.

In contrast to this, the average incubation period in thirteen monkeys inoculated by Bruce (1910) in Uganda was 12·3 days, maximum twenty-one days. Bruce was employing a virulent strain—probably directly transmitted—derived from a cattle epidemic. This strain was maintained for upwards of two years by syringe inoculation from monkey to monkey before being subjected to the transmission-experiments just referred to.

Both the *nanum* and the *congolense*-forms of this group—distinguished from one another by their different behaviour in

monkeys and dogs—have been recovered from wild game tsetse, the *nanum*-form being the most common. The *congolense*-form is acknowledged to be the more virulent, and is almost always present in cattle epidemics due to this group of trypanosomes. The evidence supplied by the experiments set forth in Section 6, taken in conjunction with the observations already recorded on this group of trypanosomes, suggests that cyclical transmission of *T. congolense* tends to the acquisition by the trypanosome of a low degree of virulence, which may be associated with inability to infect such animals as monkeys and dogs in nature. Directly transmitted natural strains, on the other hand, usually possess greater virulence, and readily infect these two animals.

SUMMARY

1. Three out of seventeen cyclical transmission experiments, performed with laboratory-bred *G. palpalis* and a wild fly strain of *T. congolense*, were successful. In the course of these seventeen experiments, one thousand and fifteen flies were dissected, of which eight hundred and ninety-three lived until the twenty-fifth day after their first feed on an infected animal; eight of these flies had flagellates established in the proboscis.

2. In all the successful transmission experiments, one or more flies with proboscis-and-gut infections had fed upon the clean animals which acquired infection. There is every reason to believe that these flies were responsible for the transmission.

3. Flies with equally intense proboscis-and-gut infections were found in two experiments in which no transmission occurred. Several of these flies had fed repeatedly on clean monkeys without infecting the animals; in no case, however, did a fly with a proboscis-and-gut infection feed upon a clean calf without infecting it. It would appear, therefore, that monkeys are less susceptible to this strain of *T. congolense*, carried by *G. palpalis*, than are calves.

4. In several cases the incubation period in the monkey was very prolonged.

5. The strain of *T. congolense* used in the experiments is less virulent than the strain used by Bruce at Mpumu, in Uganda.

The Mpumu strain was almost certainly directly transmitted before its arrival at the laboratory, while the strain here described was carried cyclically by wild tsetse. It is possible that there is a definite relation between the virulence of a strain and its method of transmission.

6. The apparent fact that the wild *G. palpalis* of the Uganda shores of Victoria Nyanza do not carry trypanosomes of the proboscis-and-gut group is to be explained, in part at any rate, by the partiality of the fly for animals which are not susceptible to this group of trypanosomes. It would appear, however, from the experiments above described, that this *G. palpalis* is less fitted to act as a true intermediate host of the *T. congolense* group of trypanosomes than of *T. vivax*, *T. uniforme*, and *T. brucei*.

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